The novel species Streptococcus tigurinus and its association with oral infection

Zbinden, A ; Bostanci, N ; Belibasakis, G N

Abstract: Streptococcus tigurinus is a novel species of viridans streptococci, shown to cause severe invasive infections such as infective endocarditis, spondylodiscitis and meningitis. S. tigurinus belongs to the Streptococcus mitis group and is most closely related to Streptococcus mitis, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pseudopneumoniae and Streptococcus infantis. The presence of S. tigurinus in the human oral cavity has been documented, including in patients with periodontal disease. This review addresses the available scientific knowledge on S. tigurinus and its association with closely related streptococci, and discusses its putative involvement in common oral infections. While there is as yet no strong evidence on the involvement of S. tigurinus with oral infections, its presence in the oral cavity and its association with endocarditis warrants special attention for a link between oral and systemic infection.

DOI: https://doi.org/10.4161/21505594.2014.970472

The novel species *Streptococcus tigurinus* and its association with oral infection.

Andrea Zbinden[1, #], Nagihan Bostanci[2], Georgios N. Belibasakis[2].

[1]Institute of Medical Virology, University of Zurich, Switzerland

[2]Institute of Oral Biology, Center of Dental Medicine, University of Zurich, Switzerland

Keywords: *Streptococcus tigurinus*, *Streptococcus mitis* group, oral infection, periodontal disease, endocarditis.

[#] **Corresponding author:** University of Zurich, Institute of Medical Virology, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Phone: +41 44 634 6213, Fax: +41 44 634 4967. E-mail: zbinden.andrea@virology.uzh.ch

Abstract

Streptococcus tigurinus is a novel species of viridans streptococci, shown to cause severe invasive infections such as infective endocarditis, spondylodiscitis and meningitis. *S. tigurinus* belongs to the *Streptococcus mitis* group and is most closely related to *Streptococcus mitis, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pseudopneumoniae* and *Streptococcus infantis*. The presence of *S. tigurinus* in the human oral cavity has been documented, including in patients with periodontal disease. This review addresses the available scientific knowledge on *S. tigurinus* and its association with closely related streptococci, and discusses its putative involvement in common oral infections. While there is as yet no strong evidence on the involvement of *S. tigurinus* with oral infections, its presence in the oral cavity and its association with endocarditis warrants special attention for a link between oral and systemic infection.

Introduction

The human oral microbiome consists of a number of bacteria; most of them are non-pathogenic commensals or act as opportunistic pathogens.[1] Some oral bacteria are implicated in oral diseases such as dental caries and periodontitis, which are among the most common infections in humans.[2, 3] Several oral bacteria have the capacity to form biofilms, which are built by complex polymicrobial mechanisms on surfaces of teeth or soft oral mucosa.[4] Viridans streptococci form a major part of the human oral microbiome, comprising four species groups: namely salivarius, anginosus, mutans and mitis group, respectively.[5] *Streptococcus mutans* is a key player in the development of dental caries,[6] and *Streptococcus anginosus* is detected in periapical odontogenic abscesses,[7] while the other species groups are less known as causative agents of oral diseases. Some bacterial species are rather associated with oral health than disease, e.g., *Streptococcus salivarius,* which is commonly found on mucosal surfaces and in the saliva.[8] Interestingly, the filamentation capacity of *S. salivarius* is markedly increased compared to other viridans streptococci, a property which may be required for a more efficient adhesion onto continuously shedding oral mucosal surfaces.[9] Other more virulent bacteria potentially enter the bloodstream, which increases the risk for invasive infections, e.g., infective endocarditis.[10] *Streptococcus mitis*, a prominent representative of viridans streptococci, is a leading cause of infective endocarditis.[11]

Recently, a novel pathogen associated with severe invasive infections such as infective endocarditis was described: *Streptococcus tigurinus* belonging to viridans streptococci.[12-14] *S. tigurinus* initially was detected in an elderly patient with infective endocarditis.[12] First, a viridans streptococcal organism was isolated in multiple blood cultures of the patient. Then, the bacterial strain was analysed by 16S rRNA gene

sequencing for accurate species identification. Analysis of the aortic valve specimen of the patient by direct 16S rRNA gene broad-range PCR[15] revealed an identical sequence compared to that of the isolate from the blood. 16S rRNA gene sequence comparison to validated sequences in the public database showed highest sequence similarity to the strain *S. mitis* ATCC 15914 with 99.9% identity (GenBank accession number AY281076). The next best match in the database was the type strain of the species *S. mitis* ATCC 49456^T (AY485601) but only with 98.6% sequence identity. Obviously, this was a novel species because of the high sequence demarcation of 1.3%. Additionally, the strain ATCC 15914, which was typed in 1977 based solely on phenotypic characteristics, must have been erroneously assigned to the species *S. mitis*.[16] Other authors have questioned the correct species assignment of strain ATCC 15914 on basis of analyses of the housekeeping genes *zwf* and *gki*[17] and the ribosomal 16S-23S intergenic spacer region.[18] Deeper analyses by different methods such as conventional biochemical testing, molecular analyses and DNA-DNA hybridization techniques proofed the presence of a novel species for which the name *S. tigurinus* was assigned.[12]

S. tigurinus is a member of the _Streptococcus mitis_ group. *S. tigurinus* belongs to the *S. mitis* group, which consists of different species, i.e., *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae, S. mitis*, *Streptococcus oralis*, *Streptococcus infantis, Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Streptococcus cristatus*, *Streptococcus gordonii*, *Streptococcus peroris, Streptococcus australis, Streptococcus oligofermentans* and *Streptococcus sinensis*.[19, 20] Recently, three novel species were assigned to the *S. mitis* group, i.e.,

Streptococcus dentisani,[21] *Streptococcus rubneri*[22] and *Streptococcus lactarius*.[23] *S. dentisani* was isolated from supragingival dental plaque from adult individuals who had never suffered from dental caries;[21] *S. rubneri* was isolated from throat swab samples of healthy humans[22] and *S. lactarius* from breast milk of healthy women.[23] Members of the *S. mitis* group are known as commensal bacteria of the human oral cavity, gastrointestinal tract and the female genital tract, however, invasive infections might occur when entering the bloodstream.[10] *S. pneumoniae* is associated with bacteremia, meningitis, otitis media and sinusitis; and is the most common cause of community-acquired pneumonia.[10] Commensals such as *S. oralis* and *S. mitis* are major pathogens for infective endocarditis in native or prosthetic heart valves.[10, 24]

Proper identification of species within the *S. mitis* group consisting of phenotypically very closely related species still remains a challenge, in particular for *S. pneumoniae*, *S. pseudopneumoniae, S. mitis*, and *S. oralis*. Identification by conventional phenotypic methods including commercial kits as VITEK 2 GP colorimetric card or API rapid 20 Strep strip (bioMérieux, Marcy-l'Etoile, France) are limited[25, 26] and newer rapid technologies such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) are useful for initial assessment to the *S. mitis* group but frequently do not allow for accurate identification.[27-29] However, there are promising reports elaborating on the differentiation of *S. pneumoniae* from other *S. mitis* group bacteria by MALDI-TOF MS.[30, 31] Molecular analysis by the 5'-part of the 16S rRNA gene, which is the gold standard for bacterial identification,[26,32-34] is not sufficiently discriminative to differentiate *S. pneumoniae*, *S. pseudopneumoniae, S. mitis* and *S. oralis* due to a >99% sequence homology.[25, 35] In the past, several other target genes like *sodA,*[36,37] *rpoB*[38] and *groEL*[39] were proposed for species differentiation. The *recA* gene was recently demonstrated as

alternative target to differentiate *S. pneumoniae* from other viridans streptococci.[40] An interspecies homology of less than 95.8% was shown within a 313-bp part of *recA,* representing a hypervariable region. Additionally, six signature nucleotides specific for *S. pneumoniae* were identified within the 313-bp *recA* fragment.

Difficulties in identifying bacteria of the *S. mitis* group to the species level might explain why the novel species *S. tigurinus* was unrecognized and underreported in the past. However, proper identification of those bacteria is important regarding species-specific pathogenicity, efficient patient management and guidance of appropriate antimicrobial therapy where needed.

Microbiological characteristics of *S. tigurinus.* *S. tigurinus* belongs to Gram-positive cocci arranged in chains and is a non-motile, non-spore-forming and catalase-negative bacterium. Colonies on sheep blood agar are smooth, white to grayish, α-hemolytic and 0.5 to 1 mm in diameter after 24 h incubation at 37°C in aerobic atmosphere. The type strain of the species is AZ_3a^T and is deposited in two different culture collections: the Culture Collection of Switzerland, CCOS, Wädenswil, Switzerland, under accession number CCOS 600^T; and the Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ, Braunschweig, Germany, under accession number DSM 24864^T. The type strain AZ_3a^T has a G+C content of the DNA of 40 mol%.[12]

Phylogenetic analysis by the 16S rRNA gene of species of the *S. mitis* group demonstrated that *S. tigurinus* type strain AZ_3a^T is clearly distinguished from the other species (Figure 1). Within the *S. mitis* group, *S. tigurinus* forms a subcluster with *S. pseudopneumoniae*, *S. pneumoniae*, *S. mitis, S. oralis* and *S. infantis* (Figure

1). The species most closely related to the *S. tigurinus* type strain AZ_3a^T is *S. mitis* AY485601 with a sequence similarity of 98.6%; the next related species are *S. infantis* AY485603 (98.5%), *S. pseudopneumoniae* AY612844 (98.3%), *S. pneumoniae* AY485600 (98.2%) and *S. oralis* AY485602 (98.1%).[12]

For laboratory diagnostic means, commercial systems such as VITEK 2 colorimetric card (bioMérieux) or MALDI-TOF MS are useful as screening method for assignment of the unknown organism into the *S. mitis* group but do not allow for accurate identification of *S. tigurinus*. Because of the limited database, analysis of the *S. tigurinus* strains by the VITEK 2 colorimetric card (bioMérieux) revealed identification as *S. mitis / S. oralis*; whereas MALDI-TOF MS analysis yielded scores of ≥ 2.2 with *S. pneumoniae,* which suggests an identification on species level.[12] Thus, for accurate identification of *S. tigurinus,* sequence analysis of the partial 16S rRNA gene is mandatory. Recently, a *S. tigurinus* specific real-time TaqMan PCR was developed for highly sensitive and specific detection of *S. tigurinus* directly in clinical samples.[41]

Ecological niche of *S. tigurinus* in the human oral microbial flora. The human oral microbiome consists of diverse bacterial phyla, e.g., *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Synergistetes* and *Proteobacteria.*[42,43] Viridans streptococci, e.g., *S. mitis*, are known to be the predominant bacterial species in the human oral cavity and were detected in various dental sites.[42] *S. oralis* is an early colonizing species of the tooth surface and can mediate the first events of biofilm formation. Thus, *S. tigurinus* was also expected to be present in the human oral microbial flora. In a previous report, *S. tigurinus* was not detected in saliva samples,

153 however, the method applied was only based on culturing methods followed by
154 analyses by MALDI-TOF MS.[13] As described before, these methods are limited in
155 accurate identification of *S. tigurinus* thus an underestimation of *S. tigurinus* in the
156 oral cavity seemed likely. By using a more sensitive method, i.e., a *S. tigurinus*
157 specific real-time TaqMan PCR, *S. tigurinus* was detected in half of the individuals
158 investigated.[41] Zbinden et al. analysed 51 saliva samples and 51 subgingival plaque
159 samples obtained from 51 individuals by the *S. tigurinus* specific real-time TaqMan
160 PCR.[41] *S. tigurinus* was detected both in saliva (n=22, 43%) and in subgingival
161 plaque samples (n=18, 35%). Overall, in 27 (53%) out of 51 individuals, *S. tigurinus*
162 was detected in the saliva samples and / or in the plaque samples. Saliva consists of
163 bacteria from different oral sites including the subgingival area. Thus it is not
164 surprising that *S. tigurinus* was found in the saliva in a higher frequency than in
165 individually selected subgingival sites. Saliva has been shown to be a suitable
166 biological fluid as alternative to pooled subgingival plaque samples for detection of
167 oral bacteria such as newly identified *Synergistetes.*[44] The presence of *S. tigurinus* in
168 the oral cavity was neither influenced by age nor nicotine consumption.[41]

169

170 **Clinical manifestation of *S. tigurinus.*** Members of the microbial flora originating
171 from the oral cavity may be involved in the pathogenesis of systemic infections.[45]
172 Biofilm formation, complex mechanisms with other bacteria or underlying diseases
173 might play a crucial role in the development of invasive infections. To date, all
174 *S. tigurinus* isolates detected from clinical human patient samples were causing
175 severe invasive infections. *S. tigurinus* most frequently caused infective endocarditis
176 (n=7).[13] Over a period of 10 years, 15% of all infective endocarditis cases caused by

viridans streptococci were caused by *S. tigurinus*.[13] Other patients developed spondylodiscitis (n=3), bacteremia (n=3), prosthetic joint infection (n=2) and meningitis (n=1) caused by *S. tigurinus*.[12-14] *S. tigurinus* was isolated from normally sterile human body sites, e.g., blood, heart valves, cerebrospinal fluid and periarticular joint biopsy specimens. All patients recovered after appropriate antimicrobial therapy, however, 8 out of 16 patients required surgical interventions. *S. tigurinus* affected not only immunocompromised patients or patients with underlying conditions such as preexisting cardiac morbidities but also healthy young patients without any comorbidities.[12-14] To date, a specific risk factor profile for the development of invasive infections with *S. tigurinus* could not be established yet due to the limited number of patients.

To note, *S. tigurinus* still might be underreported as causative agent of invasive infections for laboratory diagnostic reasons. Most microbiological diagnostic laboratories rely on identification methods by mass spectrometry, which usually allows rapid identification of bacteria but has a limited discriminative power when analysing closely related species. Molecular techniques facilitating accurate identification of *S. tigurinus* are not applicable in every routine microbiological laboratory. Yet, the occurrence of *S. tigurinus* in causing invasive infections seems geographically unlimited, since in a recent report of Japan *S. tigurinus* was demonstrated to be the causative agent in two cases of infective endocarditis.[46]

S. tigurinus is mostly fully susceptible to different antimicrobial classes. Susceptibility to penicillin, gentamicin, vancomycin, levofloxacin, erythromycin and clindamycin was demonstrated.[13] Some *S. tigurinus* strains displayed reduced susceptibility or resistance to tetracycline.[13] Nevertheless, the antibiotic resistance profile should be determined in each invasive isolate as *S. mitis* group species are known for bacterial

transformation and can acquire multiple genetic elements containing resistance genes.[47]

Association of *S. tigurinus* with oral infections. Although more than 700 species were shown to colonize the oral cavity,[42] evidence suggests that only a few of them, e.g., *Aggregatibacter actinomycetemcomitans* or *Porphyromonas gingivalis,* are associated with the pathogenesis of periodontitis or systemic complications.[48,49] Streptococci were found to be more prevalent in healthy individuals, however, *S. parasanguinis* was proposed to be involved in localized aggressive periodontitis by interaction with the major pathogen *A. actinomycetemcomitans.*[50] Furthermore, *S. mitis* is overrepresented in endodontic infections,[51] the etiology of which likely is polymicrobial.[52]

To date, it is not yet fully understood whether or not *S. tigurinus* is more prevalent in individuals with periodontitis or if it is involved in the development of other oral infections. *S. tigurinus* not only was detected in patients with periodontitis but also in individuals without periodontal diseases.[41, 53] Earlier studies have demonstrated that *S. mitis,* which is the closest related species to *S. tigurinus,* is a predominant early colonizing species of dental biofilms.[54] Although *S. mitis* is not a potent inducer of immune responses, it can antagonize the capacity of *A. actinomycetemcomitans* (a key pathogen associated with the localized aggressive form of periodontitis occurring in younger individuals) to stimulate IL-8.[55] Interaction of *S. tigurinus* with *A. actinomycetemcomitans* might be of interest.[56] Since its recent identification, it is not clear whether modifying factors are associated with the presence of *S. tigurinus*

in the human oral microbiome and if its detection in the oral cavity has direct clinical implications in systemic diseases.

Pathogenicity of *S. tigurinus.* Although *S. tigurinus* is a commensal of the human oral cavity as other members of the *S. mitis* group, specific virulence factors which allow for entering the bloodstream and causing severe invasive infections, e.g., infective endocarditis, must be present. In a rat model of experimental endocarditis, *S. tigurinus* was demonstrated to be highly virulent, however, a natural intraspecies variability of different *S. tigurinus* isolates regarding its pathogenicity potential was observed.[57] Most *S. tigurinus* strains had a more than 10-times higher capacity rate to induce aortic infection in rats than *S. gordonii*, a well-known endocarditis pathogen.[58] Moreover, the infectivity rate was similar to the most aggressive infective endocarditis pathogens, i.e., *Staphylococcus aureus* and enterococci.[57] Several virulence determinants were shown to be present in *S. tigurinus*. Phagocytosis of *S. tigurinus* by macrophages significantly was reduced compared to *S. gordonii*. The ability to resist to phagocytosis is a key attribute of invasive streptococci such as *Streptococcus pyogenes.*[59] Adherence and invasion to endothelial cells, a prerequisite for effective host cell internalization, was enhanced in some *S. tigurinus* strains as well as induction of platelet aggregation.[57] Whole-genome analyses of *S. tigurinus* were performed to unravel the genetic background of the pathogenic phenotype; genes of known virulence factors such as exfoliative toxin and fibronectin-binding protein, as well as several prophages were identified.[60]

Small-colony variants of *S. tigurinus*. The occurrence of small-colony variants (SCVs) in oral bacteria, e.g., viridans streptococci has been rather unknown. There are only a few reports describing *S. pneumoniae* mucoid variants and SCVs in biofilms.[61,62] Recently, a prosthetic joint infection caused by *S. tigurinus* SCVs was described.[14] SCVs of bacteria are a pathogenic life form promoting persistent and recurrent infections.[63] SCVs frequently cause infections associated with foreign-body material such as cardiac devices[63,64] or prosthetic joints.[65] Infection by bacterial SCVs may lead to therapy failure and often makes a definite eradication very difficult.[65-67] Therefore, timely diagnosis of these bacterial life variants has a major clinical impact regarding patient management and antibiotic therapy. Morphological and phenotypic characteristics of SCVs are small colony size, atypical colony morphology and reduced growth.[63,68] SCVs of viridans streptococci might have been overlooked in the past as even the wild-type (WT) phenotype of viridans streptococci displays tiny colonies. Moreover, overgrowth by the WT phenotype might complicate the isolation of the SCVs. SCVs often display auxotrophy for hemin, menadione or thymidine due to deficiency in electron transport or thymidine biosynthesis.[63,68] Morphological and biochemical characteristics of SCVs are extensively studied in staphylococci,[63] however, SCVs are found in various genera and species, e.g., enterococci,[69] *Escherichia coli*[70] and *Pseudomonas aeruginosa.*[63] The *S. tigurinus* SCVs displayed very small, pinpointed colonies, had a considerably reduced exponential growth phase compared to the WT and showed either a very stable or a fluctuating SCV phenotype.[14] The unstable SCV phenotype characterized by a switch from SCVs to revertant normal colonies after a few passages was first recognized in staphylococci.[64] In the *S. tigurinus* SCVs, no auxotrophy for hemin, menadione or thymidine was detected. The ultrastructure of the *S. tigurinus* SCVs were

characterized in-depth by transmission electron microscopy analyses showing major alterations in cell separation and morphological abnormalities.[14] Autolysis of *S. tigurinus* SCVs was impaired in such the SCVs displayed an increased spontaneous autolysis compared to the WT but an unexpectedly reduced autolysis under induction with Triton X-100, which is a potent autolysis inducer.[14] Whole-genome sequencing revealed mutations in genes involved in general cell metabolism, cell division, stringent response and virulence, which might partially explain the *S. tigurinus* SCV phenotype.[14]

S. pneumoniae SCVs were identified in biofilms,[61] however, whether or not *S. tigurinus* is able to develop a SCV phenotype under such circumstances is not yet known. Biofilm formation of SCV bacteria with specialized functions has been suggested to be a survival strategy, enabling tolerance to a wide variety of environmental conditions.[71] Oral bacteria forming subgingival biofilm communities may lead to development of periodontitis. In the absence of the red complex (*Treponema denticola, P. gingivalis* and *Tannerella forsythia*), *S. oralis* was found to dominate the biofilm composition in in-vitro models.[72] Hence in the absence of the red complex, *S. oralis* may display more decisive virulence characteristics within a mature biofilm community. *S. oralis* is a very closely related species of *S. tigurinus*, however, future investigations are warranted to prove if *S. tigurinus* has a similar behaviour in subgingival biofilm formation.

Conclusions

S. tigurinus is detected as part of the oral microbiota, including patients with periodontal infection. Still, a cause-effect relationship in oral infections cannot be

established by the limited data available. It further needs to be established if *S. tigurinus* can be part of pathogenic oral biofilms, how it associates with other members of the oral microbiota, and whether it is a potent inducer of pathogenic host responses. Whichever the case, however, its presence in the oral cavity and its association with endocarditis warrants special attention for a link between oral and systemic infection.

References

1. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003; 149(Pt 2):279-94.
2. Albandar JM. Underestimation of periodontitis in NHANES surveys. J Periodontol 2011; 82(3):337-41.
3. Konig J, Holtfreter B, Kocher T. Periodontal health in Europe: future trends based on treatment needs and the provision of periodontal services--position paper 1. Eur J Dent Educ 2010; 14 Suppl 1:4-24.
4. Schaudinn C, Gorur A, Keller D, Sedghizadeh PP, Costerton JW. Periodontitis: an archetypical biofilm disease. Journal of the American Dental Association 2009; 140(8):978-86.
5. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol Rev 2002; 15(4):613-30.
6. Smith EG, Spatafora GA. Gene regulation in *S. mutans*: complex control in a complex environment. J Dent Res 2012; 91(2):133-41.
7. Fisher LE, Russell RR. The isolation and characterization of milleri group streptococci from dental periapical abscesses. J Dent Res 1993; 72(8):1191-3.
8. Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, Hale JD, Wescombe PA. Influence of the probiotic *Streptococcus salivarius* strain M18 on indices of dental health in children: a randomized double-blind, placebo-controlled trial. J Med Microbiol 2013; 62(Pt 6):875-84.
9. Rossetti V, Ammann TW, Thurnheer T, Bagheri HC, Belibasakis GN. Phenotypic diversity of multicellular filamentation in oral streptococci. PLoS One 2013; 8(9):e76221.

10. Spellerberg B, Brandt C. *Streptococcus*. In: Versalovic J, Carroll KC, Jorgensen JH, Funke G, Landry ML, Warnock DW, eds. Manual of clinical microbiology. Washington DC: American Society for Microbiology, 2011:331-49.
11. Hoen B, Alla F, Selton-Suty C, Beguinot I, Bouvet A, Briancon S, Casalta JP, Danchin N, Delahaye F, Etienne J, et al. Changing profile of infective endocarditis: results of a 1-year survey in France. JAMA 2002; 288(1):75-81.
12. Zbinden A, Mueller NJ, Tarr PE, Sproer C, Keller PM, Bloemberg GV. *Streptococcus tigurinus* sp. nov., isolated from blood of patients with endocarditis, meningitis and spondylodiscitis. Int J Syst Evol Microbiol 2012; 62:2941-5.
13. Zbinden A, Mueller NJ, Tarr PE, Eich G, Schulthess B, Bahlmann AS, Keller PM, Bloemberg GV. *Streptococcus tigurinus*, a novel member of the *Streptococcus mitis* group, causes invasive infections. J Clin Microbiol 2012; 50(9):2969-73.
14. Zbinden A, Quiblier C, Hernandez D, Herzog K, Bodler P, Senn MM, Gizard Y, Schrenzel J, Francois P. Characterization of *Streptococcus tigurinus* small-colony variants causing prosthetic joint infection by comparative whole-genome analyses. J Clin Microbiol 2014; 52(2):467-74.
15. Bosshard PP, Kronenberg A, Zbinden R, Ruef C, Boettger EC, Altwegg M. Etiologic diagnosis of infective endocarditis by broad-range polymerase chain reaction: a 3-year experience. Clin Infect Dis 2003; 37(2):167-72.
16. Facklam RR. Physiological differentiation of viridans streptococci. J Clin Microbiol 1977; 5(2):184-201.

17. Kiratisin P, Li L, Murray PR, Fischer SH. Use of housekeeping gene sequencing for species identification of viridans streptococci. Diagn Microbiol Infect Dis 2005; 51(4):297-301.
18. Tung SK, Teng LJ, Vaneechoutte M, Chen HM, Chang TC. Identification of species of *Abiotrophia*, *Enterococcus*, *Granulicatella* and *Streptococcus* by sequence analysis of the ribosomal 16S-23S intergenic spacer region. J Med Microbiol 2007; 56(Pt 4):504-13.
19. Hoshino T, Fujiwara T, Kilian M. Use of phylogenetic and phenotypic analyses to identify nonhemolytic streptococci isolated from bacteremic patients. J Clin Microbiol 2005; 43(12):6073-85.
20. Tong H, Gao X, Dong X. *Streptococcus oligofermentans* sp. nov., a novel oral isolate from caries-free humans. Int J Syst Evol Microbiol 2003; 53(Pt 4):1101-4.
21. Camelo-Castillo A, Benitez-Paez A, Belda-Ferre P, Cabrera-Rubio R, Mira A. *Streptococcus dentisani* sp. nov., a novel member of the mitis group. Int J Syst Evol Microbiol 2014; 64(Pt 1):60-5.
22. Huch M, De Bruyne K, Cleenwerck I, Bub A, Cho GS, Watzl B, Snauwaert I, Franz CM, Vandamme P. *Streptococcus rubneri* sp. nov., isolated from the human throat. Int J Syst Evol Microbiol 2013; 63(Pt 11):4026-32.
23. Martin V, Manes-Lazaro R, Rodriguez JM, Maldonado-Barragan A. *Streptococcus lactarius* sp. nov., isolated from breast milk of healthy women. Int J Syst Evol Microbiol 2011; 61(Pt 5):1048-1052.
24. Douglas CW, Heath J, Hampton KK, Preston FE. Identity of viridans streptococci isolated from cases of infective endocarditis. J Med Microbiol 1993; 39(3):179-82.

25. Arbique JC, Poyart C, Trieu-Cuot P, Quesne G, Carvalho Mda G, Steigerwalt AG, Morey RE, Jackson D, Davidson RJ, Facklam RR. Accuracy of phenotypic and genotypic testing for identification of *Streptococcus pneumoniae* and description of *Streptococcus pseudopneumoniae* sp. nov. J Clin Microbiol 2004; 42(10):4686-96.

26. Bosshard PP, Abels S, Altwegg M, Boettger EC, Zbinden R. Comparison of conventional and molecular methods for identification of aerobic catalase-negative Gram-positive cocci in the clinical laboratory. J Clin Microbiol 2004; 42(5):2065-73.

27. van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 2010: 48(3):900-7.

28. Ferroni A, Suarez S, Beretti JL, Dauphin B, Bille E, Meyer J, Bougnoux ME, Alanio A, Berche P, Nassif X. Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010; 48(5):1542-8.

29. Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Boettger EC, Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. J Clin Microbiol 2013; 51(6):1834-40.

30. Werno AM, Christner M, Anderson TP, Murdoch DR. Differentiation of *Streptococcus pneumoniae* from nonpneumococcal streptococci of the

403 *Streptococcus mitis* group by matrix-assisted laser desorption ionization-time
404 of flight mass spectrometry. J Clin Microbiol 2012; 50(9):2863-7.
405 31. Branda JA, Markham RP, Garner CD, Rychert JA, Ferraro MJ. Performance of
406 the Vitek MS v2.0 system in distinguishing *Streptococcus pneumoniae* from
407 nonpneumococcal species of the *Streptococcus mitis* group. J Clin Microbiol
408 2013; 51(9):3079-82.
409 32. Boettger EC. Approaches for identification of microorganism. ASM news 1996;
410 61:247-50.
411 33. Clarridge JE, 3rd. Impact of 16S rRNA gene sequence analysis for
412 identification of bacteria on clinical microbiology and infectious diseases. Clin
413 Microbiol Rev 2004; 17(4):840-62, table of contents.
414 34. Rogall T, Flohr T, Boettger EC. Differentiation of *Mycobacterium* species by
415 direct sequencing of amplified DNA. J Gen Microbiol 1990; 136(9):1915-20.
416 35. Kawamura Y, Hou XG, Sultana F, Miura H, Ezaki T. Determination of 16S
417 rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and
418 phylogenetic relationships among members of the genus *Streptococcus*. Int J
419 Syst Bacteriol 1995; 45(2):406-8.
420 36. Kawamura Y, Whiley RA, Shu SE, Ezaki T, Hardie JM. Genetic approaches to
421 the identification of the mitis group within the genus *Streptococcus*.
422 Microbiology 1999; 145 (Pt 9):2605-13.
423 37. Poyart C, Quesne G, Coulon S, Berche P, Trieu-Cuot P. Identification of
424 streptococci to species level by sequencing the gene encoding the
425 manganese-dependent superoxide dismutase. J Clin Microbiol 1998; 36(1):41-
426 7.

427 38. Drancourt M, Roux V, Fournier PE, Raoult D. *rpoB* gene sequence-based
428 identification of aerobic Gram-positive cocci of the genera *Streptococcus*,
429 *Enterococcus*, *Gemella*, *Abiotrophia*, and *Granulicatella*. J Clin Microbiol 2004;
430 42(2):497-504.
431 39. Glazunova OO, Raoult D, Roux V. Partial sequence comparison of the *rpoB*,
432 *sodA*, *groEL* and *gyrB* genes within the genus *Streptococcus*. Int J Syst Evol
433 Microbiol 2009; 59(Pt 9):2317-22.
434 40. Zbinden A, Kohler N, Bloemberg GV. A *recA* based PCR assay for accurate
435 differentiation of *Streptococcus pneumoniae* from other viridans streptococci. J
436 Clin Microbiol 2011; 49:523-7.
437 41. Zbinden A, Aras F, Zbinden R, Mouttet F, Schmidlin PR, Bloemberg GV,
438 Bostanci N. Frequent detection of *Streptococcus tigurinus* in the human oral
439 microbial flora by a specific 16S rRNA gene real-time TaqMan PCR. BMC
440 Microbiol 2014; Aug 24;14(1):231. [Epub ahead of print].
441 42. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal
442 bacterial flora of the oral cavity. J Clin Microbiol 2005; 43(11):5721-32.
443 43. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, Lakshmanan A,
444 Wade WG. The human oral microbiome. J Bacteriol 2010; 192(19):5002-17.
445 44. Belibasakis GN, Ozturk VO, Emingil G, Bostanci N. *Synergistetes* cluster A in
446 saliva is associated with periodontitis. J Periodontal Res 2013; 48(6):727-32.
447 45. Deshpande RG, Khan M, Genco CA. Invasion strategies of the oral pathogen
448 *Porphyromonas gingivalis*: implications for cardiovascular disease. Invasion
449 Metastasis 1998; 18(2):57-69.

46. Miyazato A, Ohkusu K, Tachi Y, Hashikita G, Ezaki T, Mitsutake K. Two cases of infective endocarditis caused by *Streptococcus tigurinus*. Kansenshogaku Zasshi 2014; 88(3):304-6.

47. Bek-Thomsen M, Poulsen K, Kilian M. Occurrence and evolution of the paralogous zinc metalloproteases IgA1 protease, ZmpB, ZmpC, and ZmpD in *Streptococcus pneumoniae* and related commensal species. MBio 2012; 3(5):e00303-12.

48. Papapanou PN, Behle JH, Kebschull M, Celenti R, Wolf DL, Handfield M, Pavlidis P, Demmer RT. Subgingival bacterial colonization profiles correlate with gingival tissue gene expression. BMC Microbiol 2009; 9:221.

49. Socransky SS, Haffajee AD. Implications of periodontal microbiology for the treatment of periodontal infections. Compend Suppl 1994; (18):S684-685, 688-693; quiz S714-687.

50. Fine DH, Markowitz K, Fairlie K, Tischio-Bereski D, Ferrendiz J, Furgang D, Paster BJ, Dewhirst FE. A consortium of *Aggregatibacter actinomycetemcomitans*, *Streptococcus parasanguinis*, and *Filifactor alocis* is present in sites prior to bone loss in a longitudinal study of localized aggressive periodontitis. J Clin Microbiol 2013; 51(9):2850-61.

51. Hsiao WW, Li KL, Liu Z, Jones C, Fraser-Liggett CM, Fouad AF. Microbial transformation from normal oral microbiota to acute endodontic infections. BMC Genomics 2012; 13:345.

52. do Cabo Fernandes C, Rechenberg DK, Zehnder M, Belibasakis GN. Identification of *Synergistetes* in endodontic infections. Microbial pathogenesis 2014; 73C:1-6.

53. Dhotre SV, Mehetre GT, Dharne MS, Suryawanshi NM, Nagoba BS. Isolation of *Streptococcus tigurinus* - a novel member of *Streptococcus mitis* group from a case of periodontitis. FEMS Microbiol Lett 2014; 357(2):131-5.
54. Li J, Helmerhorst EJ, Leone CW, Troxler RF, Yaskell T, Haffajee AD, Socransky SS, Oppenheim FG. Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol 2004; 97(6):1311-8.
55. Sliepen I, Van Damme J, Van Essche M, Loozen G, Quirynen M, Teughels W. Microbial interactions influence inflammatory host cell responses. J Dent Res 2009; 88(11):1026-30.
56. Ennibi OK, Benrachadi L, Bouziane A, Haubek D, Poulsen K. The highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans* in localized and generalized forms of aggressive periodontitis. Acta Odontol Scand 2012; 70(4):318-22.
57. Veloso TR, Zbinden A, Andreoni F, Giddey M, Vouillamoz J, Moreillon P, Zinkernagel AS, Entenza JM. *Streptococcus tigurinus* is highly virulent in a rat model of experimental endocarditis. Int J Med Microbiol 2013; 303(8):498-504.
58. Bizzini A, Beggah-Moller S, Moreillon P, Entenza JM. Lack of in vitro biofilm formation does not attenuate the virulence of *Streptococcus gordonii* in experimental endocarditis. FEMS Immunol Med Microbiol 2006; 48(3):419-23.
59. Courtney HS, Hasty DL, Dale JB. Anti-phagocytic mechanisms of *Streptococcus pyogenes*: binding of fibrinogen to M-related protein. Mol Microbiol 2006; 59(3):936-47.
60. Gizard Y, Zbinden A, Schrenzel J, Francois P. Whole-genome sequences of *Streptococcus tigurinus* type strain AZ_3a and *S. tigurinus* 1366, a strain

causing prosthetic joint infection. Genome Announcements 2013; 1(2):e00210-2.

61. Allegrucci M, Sauer K. Characterization of colony morphology variants isolated from *Streptococcus pneumoniae* biofilms. J Bacteriol 2007; 189(5):2030-8.

62. Allegrucci M, Sauer K. Formation of *Streptococcus pneumoniae* non-phase-variable colony variants is due to increased mutation frequency present under biofilm growth conditions. J Bacteriol 2008; 190(19):6330-9.

63. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 2006; 4(4):295-305.

64. Maduka-Ezeh A, Seville MT, Kusne S, Vikram HR, Blair JE, Greenwood-Quaintance K, Arabia F, Patel R. Thymidine auxotrophic *Staphylococcus aureus* small-colony variant endocarditis and left ventricular assist device infection. J Clin Microbiol 2012; 50(3):1102-5.

65. Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W. *Staphylococcus aureus* small colony variants in prosthetic joint infection. Clin Infect Dis 2006; 43(8):961-7.

66. Vaudaux P, Kelley WL, Lew DP. *Staphylococcus aureus* small colony variants: difficult to diagnose and difficult to treat. Clin Infect Dis 2006; 43(8):968-70.

67. von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G. Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease. Clin Infect Dis 2001; 32(11):1643-7.

68. Kahl BC, Belling G, Reichelt R, Herrmann M, Proctor RA, Peters G. Thymidine-dependent small-colony variants of *Staphylococcus aureus* exhibit

gross morphological and ultrastructural changes consistent with impaired cell separation. J Clin Microbiol 2003; 41(1):410-3.

69. Groebner S, Beck J, Schaller M, Autenrieth IB, Schulte B. Characterization of an *Enterococcus faecium* small-colony variant isolated from blood culture. Int J Med Microbiol 2012; 302(1):40-4.

70. Sendi P, Frei R, Maurer TB, Trampuz A, Zimmerli W, Graber P. *Escherichia coli* variants in periprosthetic joint infection: diagnostic challenges with sessile bacteria and sonication. J Clin Microbiol 2010; 48(5):1720-5.

71. Boles BR, Thoendel M, Singh PK. Self-generated diversity produces "insurance effects" in biofilm communities. Proc Natl Acad Sci USA 2004; 101(47):16630-5.

72. Thurnheer T, Belibasakis GN, Bostanci N. Colonisation of gingival epithelia by subgingival biofilms in vitro: Role of "red complex" bacteria. Archives of oral biology 2014; 59(9):977-86.

Figure 1. Phylogenetic analysis of the *Streptococcus mitis* group.

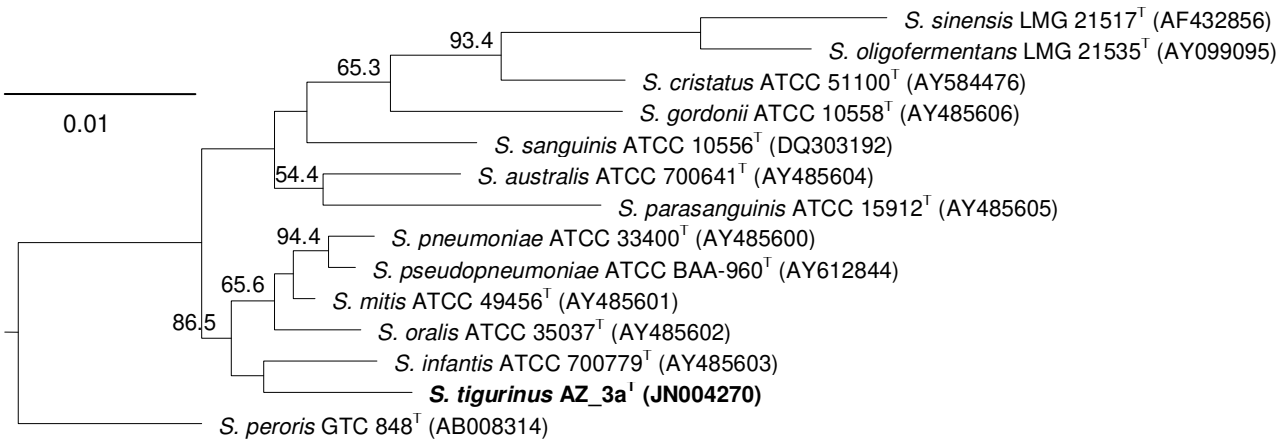

Figure legend 1.

The neighbour-joining phylogenetic tree based on partial 16S rRNA gene sequences (>1300 bp) shows the relationships among *S. tigurinus* strain AZ_3a^T and related species within the *S. mitis* group. Bootstrap percentages (based on 1000 replications) > 50% are shown at branching points. Published sequences used were from the public GenBank database. Bar, 0.01 substitutions per nucleotide position.